## WS4.1 New molecular models of CFTR based on ABC transporter structures

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CFTR is a chloride channel belonging to the ABC superfamily. In previous work, we have developed models of the whole 3D structure of human CFTR based on experimental structures of proteins belonging to the ABC superfamily, in outward and inward-facing conformations (Sav1866, Msba) (Mornon 2008 & 2009, Serohijos 2008). These models, together with molecular dynamics (MD) simulations based on Sav1866 (Norimatsu 2012, Dalton 2012) and PgP (Furukawa 2013), were used to understand the molecular basis of the CFTR functioning.

Here, we present a refined sequence alignment and a model of the CFTR open channel which uses information from all five known 3D structures of ABC exporters. This model provides a new vision of the pore and the interfaces of the intracellular loops 1 and 3 with the NBDs. We also describe a new model of CFTR based on the structure of the heterodimeric ABC transporter TM287-TM288 in an inward-facing state. As CFTR, this structure has nonequivalent ATP-binding sites. The two NBDs only partially separate, remaining in contact through an interface involving conserved motifs that connect the two ATP-binding sites. Thus, this new model of the CFTR closed channel allows unprecedented insights into the role of specific amino acids, typifying the CFTR noncanonical ATP-binding site. MD has been performed in order to relax the model and obtain a structure probably closer to reality. The refined models presented here pave the way for a search of molecules able to fix the assembly of the protein and its thermal stability, a major cause of the low ability of CFTR to reach and stay at the plasma membrane.

Supported by the French Association Vaincre La Mucoviscidose Paris.

## WS4.2 Exploration of the ATP binding site in CFTR-NBD1 for enhanced ligand binding

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Symptomatic treatments exist for the most common CF-genotype, F508del, but the utility of CF transmembrane conductance regulator (CFTR) as a target, has been demonstrated by the efficacy of a direct molecular modulator of patients with the G551D mutation. The F508del mutation reduces folding efficiency and thermodynamic stability of the first nucleotide binding domain (NBD1) and also interferes with interactions of NBD1 with other CFTR domains, e.g. ICDs.

Recent studies show that suppressor mutations, when expressed on the F508del-NBD1 background, are able to enhance the thermal stability of the mutant domain and to partially rescue ΔF508-CFTR. These results suggest that ΔF508-NBD1 is a viable target for ΔF508-CFTR rescuing through the pharmaceutical intervention of CFTR modulators binding to specific sites on this domain. Detailed computational analysis of the characteristics of such sites may lead to identification of drug-like compounds that may ultimately rescue ΔF508-CFTR.

Here we describe a computational methodology for predicting the binding affinity of potential modulators to the ATP binding site of wildtype and mutant CFTR NBD1. First we dock a selected set of ATP analogues into the ATP site of wildtype (1R0X) and mutant (1XMG) NBD1 by means of in-site conformational search. Next, the lowest energy pose of each analogue is selected and further scored by its ability to form favorable interactions with binding site residues in accord with a pre-suggested binding hypothesis. Our affinity predictions were validated by melting temperature ($T_M$) analysis of NBD1-ligand. The proposed methodology could therefore be applied to the design new CFTR modulators.

## WS4.3 Rescue of F508del-CFTR without the regulatory insertion (ΔRI) and regulatory extension (ΔRE) in combination with genetic revertants and small molecules

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In the absence of high-resolution structures for CFTR, deletion mutants are highly relevant to understand how certain protein regions affect CFTR maturation and function. The presence of two NBD1 regions which are absent from other ABC transporters – the regulatory extension (RE) and regulatory insertion (RI) – shown to be conformationally dynamic in F508del-NBD1-CFTR and more hidden in wt-NBD1-CFTR, may contribute to the low folding efficiency of the mutant through exposure of hydrophobic surfaces.

Our goal was to assess the impact of removing RE and RI (separately and jointly) on the *in vivo* processing and function of full-length wt- and F508del-CFTR.

Our data show that removal of RI alone, or together with RE, from F508del-CFTR restores both maturation and function to the levels of wt-CFTR, suggesting that these variants acquire a native (fully folded) conformation *in vivo*. We also evaluated the effect of known F508del-revertant mutations (R1070W, G550E) on maturation of ΔRE and ΔRI variants. Both revertants failed to rescue F508del-ΔRE-CFTR processing, but further increase F508del-ΔRI-CFTR processing. VX-809 did not significantly rescue ΔRE, but it significantly increased rescue of all ΔRI-F508del-CFTR variants. Most of the variants respond less to VX-770 than to Genistein as a channel potentiator.

Additional studies on maturation efficiency and functional characterization of these CFTR and other ΔRE/ΔRI variants with CFTR modulators are underway to further elucidate their impact on CFTR structure.

Supported by PTDC/SAU-GMG/122299/2010 grant (POCTI/FCT/PIDDAC, Portugal), PEst-OE/BIA/UI4046/2011 centre grant (to BioFIG) and FCT/SFRH/BD/69180/2010 PhD fellowship.

## WS4.4 Restoration of F508Δ-CFTR trafficking and function by liposome-mediated delivery of antibodies against cytokeratin 8

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Cystic Fibrosis (CF) is due to mutations in the gene encoding the CFTR ion channel. The most common is the deletion of a phenylalanine in position 508 (ΔF508-CFTR). This results in a channel which is partially functional but is retained and degraded in the endoplasmic reticulum (ER) due to a defect in the trafficking towards plasma membrane. It was previously shown that cytokeratin 8 (K8) level is increased in cells expressing ΔF508-CFTR (Davezac et al., Proteomics 2004), and a more recent study showed that K8 strongly interacts with ΔF508-CFTR, contributing to the retention of the channel in the ER. Furthermore, it was observed that K8 ablation by siRNA led to restoration of CFTR-dependent conductance *in vitro* and *in vivo*, suggesting that K8 is a potential therapeutic target in CF (Colas et al., Hum.Mol.Genet. 2012). We hypothesized that the intracellular delivery of antibodies directed against K8 into ΔF508-CFTR expressing cells could also disrupt the interaction between K8 and mutant CFTR, and restore its traffic.

We developed a novel liposome-based system for the delivery of proteins into living cells and showed its ability to deliver an antibody directed against K8, leading to the labeling of the keratin cytoskeleton into living cells. By monitoring halide efflux in treated cells, we observed the appearance of a conductance compatible with CFTR activity, suggesting the ability of the delivered antibodies to disrupt the interaction between K8 and F508Δ-CFTR and consequently to restore the traffic of F508Δ-CFTR.

This original protein-based strategy could provide a new tool to CF researchers and contribute to the development of biotherapies against this pathology.